UNIVERSITY OF CALIFORNIA,
IRVINE

Clinical Improvement Mirrored Antibody Reduction in Myasthenia Gravis

THESIS

submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Biomedical and Translational Sciences

by

Isela Stephanie Hernandez

Thesis Committee:
Professor Tahseen Mozaffar, Chair
Professor Sherrie Kaplan
Associate Professor Ali A. Habib

2022

DEDICATION

To

- To my Loving Parents: Mom and Papa – Thank you for your endless sacrifices, and never-ending support. You both have allowed this dream to be a reality, and I am eternally grateful for you both.

- To my Best Friends/Sisters: Angela, Nataly, Sabrina, & Stephanie - Thank you for always being my greatest supporters and for always believing in me (even when I didn't).

- To my Partner/Best Friend: Danny - Thank you for always being by my side through the highs and lows, your love and patience will never go unappreciated.

- To my Colleagues: The Fantastic 4 (Josh, Shayda, Violeta) & My New Friend (Amanda) - Thank you for the laughs, sleepless nights, and tremendous support. This journey wouldn't have been possible or as gratifying without you all.

- To my Professors/Counselors: Dr. Aban, Dr. Fan, Dr. Greenfield, Dr. Goyal, Dr. Kelly, Dr. Shin, Dr. Wilson, Marissa Saplala, and Thuy Pham– Thank you for your knowledge, endless guidance, and support.

- To my Mentors: Dr. Mozaffar, Dr. Kaplan, & Dr. Habib - Thank you for your great knowledge, guidance, and for challenging my scientific mind.

- And a Special thank you to my MG patients - Thank you for sharing your journeys, as your stories have inspired me to further our understanding and knowledge about myasthenia gravis.

This thesis is dedicated to you all, for the completion of this journey came to fruition because of you.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

MG	: Myasthenia Gravis
MG-ADL	: Myasthenia Gravis Activities of Daily Living
QMG:	Quantitative Myasthenia Gravis
AChR	: anti-acetylcholine receptor
MGTX	: Thymectomy Trial in Non-Thymomatous Myasthenia Gravis Patients Receiving Prednisone Therapy
ΔMG-ADL	: Changes in Myasthenia Gravis Activities of Daily Living score
ΔQMG	: Changes in Quantitative Myasthenia Gravis score
ΔAb	: Changes in AChR antibody level
ETTX	: Extended transsternal thymectomy
MGFA	: Myasthenia Gravis Foundation of America

ACKNOWLEDGEMENTS

I would like to express the deepest appreciation to my committee chair, Professor Dr. Tahseen Mozaffar, and my committee members, Dr. Sherrie Kaplan and Dr. Ali A. Habib. For their great knowledge, guidance, and for challenging my scientific mind. Without their guidance and persistent help this thesis would not have been possible.

In addition, a thank you to Dr. Fan, Dr. Greenfield, Dr. Goyal, Dr. Kelly, Dr. Shin, Dr. Wilson, Marissa Saplala, and Thuy Pham. Thank you for your knowledge, endless guidance, and support.

Lastly, I would like to extend my deep appreciation to the Investigators of the MGTX clinical trial for their permission to utilize their data for my thesis. Thank you, Dr. Gil I. Wolfe, Dr. Henry Kaminski, Dr. Gary Cutter, Dr. Inmaculada Aban, and Dr. Angela Vincent.

ABSTRACT OF THE THESIS

Clinical Improvement Mirrored Antibody Reduction in Myasthenia Gravis

by

Isela Stephanie Hernandez

Master of Science in Biomedical and Translational Science

University of California, Irvine, 2022

Professor Tahseen Mozaffar, Chair

Introduction: The relationship of anti-acetylcholine receptor (AChR) antibody levels to treatment response remains unclear in seropositive myasthenia gravis (MG) patients.

Objective: To examine whether changes in AChR antibody level (ΔAb) correlate with clinical response in subjects in the Thymectomy in Myasthenia Gravis Trial (MGTX).

Methods: Post-hoc analysis of the MGTX antibody level dataset at baseline, 12, 24, 36 months. Changes in Myasthenia Gravis Activities of Daily Living (ΔMG-ADL) and Quantitative Myasthenia Gravis (ΔQMG) scores compared to ΔAb between the thymectomy+prednisone versus prednisone only groups. Statistical methods included bivariate linear regression, Spearman correlation and Mann-Whitney test.

Results: Data from 86/126 enrolled subjects, including outliers, was analyzed. Correlation with ΔMG-ADL was statistically significant at 12 and 24 months (P 0.0397 and 0.0008 respectively). ΔQMG and ΔAb directly correlated at all 3 timepoints [P= 0.0032, P= 0.0031, P=0.0005, respectively].

Conclusion: Reductions in AChR antibody level generally correlated, in both treatment arms, with improvement in QMG and MG-ADL scores, confirming the utility of monitoring antibody levels in clinical practice to follow treatment response.

CHAPTER 1: INTRODUCTION

Myasthenia Gravis and Anti-Acetylcholine Receptor Antibody Levels

Myasthenia gravis (MG) is a rare neuromuscular disease caused by a defect in the neuromuscular transmission. [2] Koneczny and Herbst confirmed that autoantibodies, anti–acetylcholine receptor (AChR) antibody (Ab), are present in approximately 85% of MG patients. [15] The nicotinic acetylcholine receptor are the key molecules at the neuromuscular junction that AChR Ab target, which result in reduced neuromuscular transmission. [15] Once the MG autoantibodies target the neuromuscular junction, neuromuscular transmission is reduced, resulting in severe muscle weakness and fatigability. [9] The most common symptoms and signs at onset of myasthenia gravis are: ptosis, dipoplia, dysarthria, and weakness in the face, neck, upper limbs, and lower limbs.[2]

Currently, a cure still does not exist for myasthenia gravis, but there are several treatments offered to help manage MG symptoms. Some of these treatments include immunomodulatory drugs, plasma exchange, symptomatic pharmacological treatment, and supportive therapies. [9] Although not a cure, the listed treatments allow MG patients to live a better quality of life. Nonetheless, managing the treatments offered for myasthenia gravis has its limitations, as clinicians cannot rely on a guide, such as a biomarker for disease severity. As discussed by Carr *et al.,* their systemic review of population based epidemiological studies in MG from 1950 to 2007, revealed that myasthenia gravis continues to be one of the most predominant (7.8/100,000) neuromuscular disorders of neuromuscular transmission. [4] As MG continues to be the most prevalent neuromuscular disorder of neuromuscular transmission, furthering our scientific knowledge on a clinical guide, such as a biomarker for disease severity, is essential.

Previous Research: Antibody Levels and MG Clinical Status

Today, anti-acetylcholine receptor antibody levels are purely used for establishing myasthenia gravis diagnosis. The use of AChR antibodies as a biomarker for clinical status in MG patients continues to be a controversial topic, as correlation of AChR antibody level and MG treatment outcomes has been scarcely investigated. In 1976, Lindstrom *et al.* discovered that antibody levels did not correlate with sex, age, steroid therapy, or duration of symptoms in MG patients. [18] Additionally, these investigators found that a correlation existed between the presence of antibody titers and ocular muscle weakness and presence of thymoma in MG patients.[18]

The correlation of antibody level and MG clinical status continued to evolve. In 1981, Seybold *et al.* discovered that antibody levels that decreased more than 50% over several months, generally associated with clinical status improvement in MG patients.[22] These investigators argued that serial AChR antibody titers were important for the management of MG treatment, but only for severely affected MG patients. [22]

Seybold and colleagues argued that antibody titers were only important for severely affected MG patients, and not moderately affected MG patients. However, Howard *et al.* study data demonstrated that MG patients with severe clinical classification generally demonstrated higher levels of AChR antibody titers, but MG patients with severe clinical status also demonstrated low antibody levels[12] This discovery implied that antibody levels did not correlate with MG clinical status, as the researchers found high and low antibody levels in severely affected MG patients.

Researchers continued to investigate if an association existed between AChR antibody levels and MG clinical status. In 1992, Somnier *et al.* emphasized that the

correlation between clinical parameters and AChR antibodies is complicated, but their data showed that generalized MG and females had higher titer levels, compared to ocular MG and males. [21] Additionally, Somnier and colleagues concluded that low to intermediate titers correlated with normal thymus, as where hyperplastic thymus correlated with high levels of antibodies.[21]

Subsequently, in 2009, Aurangzeb and colleagues conducted a prospective study to analyze the relationship of MG severity and anti-acetylcholine receptor antibody titers. They found that the antibody titers did not correlate with the clinical severity of MG patients.[1] Although the association between clinical status and antibody levels was not statistically significant, the Investigators identified that AChR antibody levels were higher in women, compared to men, and higher in younger age groups. [1] Similarly, Sanders *et al.* reviewed data of 86 MG patients in a prospective trial in 2014 and established that AChR antibody levels should not be utilized as a biomarker for improvement in MG.[21] The researchers' recommendation was a result of their analysis, which showed that reductions in AChR antibody levels were seen in both improved and unimproved patients. [21]

Correspondingly, in 2018, Giuliana and colleagues surveyed a cohort of 175 Caucasian MG patients and concluded that worse clinical outcome was not related to the AChR titer level. [8] Although study limitations were not identified in this study, only Caucasian patients were analyzed. Sampling bias is a potential study limitation, as only individuals of the same ethnic background were recruited for this study, thus resulting in a biased sample population. Contrary to the three previous investigators, Kang *et al.* investigated the clinical severity of MG patients to AChR antibody type. After conducting the retrospective study, Investigators disclosed that when both binding and blocking

antibodies were present, patients demonstrated severe generalized MG. [13] The Investigators acknowledged that limitations existed in their research, one of the greatest limitations was sample size: this study only included 35 patients. [13]

In 2014, Heldal and colleagues conducted a prospective study in 67 MG patients and found a positive association between MGFA clinical classification score decline and AChR-antibody level concentration over time. [10] This association was only seen in the immunosuppressive treatment group, but not in the pyridostigmine only study group.[10] Additionally, the Investigators disclosed that only a single laboratory for the AChR-antibody assays was performed, and that the AChR-antibody sample were considered a valid sample if captured 1 month before or 1 month after the MGFA score was obtained. [10] If antibody samples captured 1 month before or after were considered valid samples, a large gap in time is introduced, meaning that the antibody samples that were collected may not potentially be the most accurate representation of the MG patients' clinical status.

Within the last few years, limited publications have argued that a correlation exists between AChR antibody levels and MG clinical status. In 2019, Cheng-Che and colleagues conducted a retrospective study analyzing 54 juvenile MG patients. These researchers found that patients without AChR antibodies demonstrated an increase in complete remission rates, compared to patients with present antibodies. [6] The investigators identified several limitations in their study, one of the greatest limitations was that antibodies were not measured at the same timepoints for all patients, and that some of the antibodies were calculated post-immune therapy.[6] Issues with collecting the serum antibody sample and clinical status at the same timepoint continues to be a flaw in these previous studies.

Similarly, Yuta and colleagues conducted a retrospective study analyzing 53 AChR antibody positive MG patients. Antibody levels were measured within 100 days of initiating immunosuppressive treatment, and clinical status was assessed using the MGFA post-intervention status and MG activity of daily living (MG-ADL) at 1-year post treatment. [14] They found that higher AChR level reduction rates had lower MG-ADL scores and a higher ratio of minimal manifestations, compared to the study group with lower AChR level reduction rate.[14] Yuta *et al.* concluded that higher reduction rates in AChR level were associated with favorable outcomes 1-year post immunosuppressive treatment and improvement of MG symptoms.[14] Collection of antibody sample and MG patient's clinical evaluation at the same timepoint continues to be an issue seen in these previously conducted studies. With the constant fluctuations in MG symptoms, antibody levels that are not captured at the same timepoint as the clinical evaluation may not be the most accurate representation of the patients' clinical status. Thus, antibody samples and the MG clinical status should be captured at the same timepoint. Alongside collecting the antibody sample and clinical status at different timepoints, the investigators identified that other limitations within the research were that data for the MG-ADL was missing for several patients[14] Recently published in 2021, Marcuse and colleagues conducted a retrospective study and their analysis showed that a change in AChR antibody level is associated with MG clinical status. [19] They demonstrated an inverse association between MGFA improvement and change in AChR antibody level.[19] Ninety MG patients' charts were retrospectively reviewed from1997 to 2020 and a blinded clinician determined MGFA classification using the electronic patient file.[19] Marcuse *et al.* argued that AChR antibody levels could potentially be biomarkers for clinical improvement in MG patients. [19]

Previous research has attempted to investigate the association between AChR antibody levels and MG clinical status, but the methodology of these studies has demonstrated various flaws. One of the greatest flaws seen has been that AChR antibody levels and clinical status have been collected at different timepoints, resulting in antibody samples potentially not being the most accurate representation of MG patients' clinical status. Nonetheless, a guide to help manage treatments for MG patients continues to persist. The use of AChR antibody levels as a biomarker for clinical status may help guide clinicians in managing the treatments offered to MG patient.

Specific Aims

Correlation of AChR antibody level with MG treatment outcomes has not been systematically studied in relation to thymectomy. This research will utilize the data from the phase III clinical trial- titled: A Multi-Center, Single-Blind, Randomized Study Comparing Thymectomy to No Thymectomy in Non-Thymomatous Myasthenia Gravis (MG) Patients Receiving Prednisone (MGTX). Through post-hoc analysis of the MGTX controlled clinical trial database, this research may potentially further our scientific knowledge. To date, anti-acetylcholine receptor antibody levels are used purely for establishing myasthenia gravis diagnosis. The overall objective of this research is to utilize this post-hoc analysis to examine whether changes in AChR antibody level (ΔAb) correlate with clinical response through changes in MG-ADL (ΔMG-ADL) and changes in QMG (ΔQMG) scores. This research will strive to determine the association between MG patients study group (ETTX vs. prednisone alone) and the percent change in AChR antibody levels from baseline to 12, 24, and 36 months. This study will compare the percent change in antibody levels of patients from their baseline visit to their 12, 24, and 36 months visit. It is hypothesized that patients randomized to the thymectomy (ETTX) plus prednisone group will demonstrate greater reductions in antibody levels at 12, 24, and 36 months, compared to patients in the control group (prednisone alone). Secondly, this research aims to determine the association between percent change in acetylcholine receptor antibody levels from baseline to 12, 24, 36 months and the change in MG-ADL and QMG score at the corresponding timepoints. It is hypothesized that greater reductions in antibody levels will result in clinical improvement (decrease in scores) in MG-ADL and QMG scores over time.

Overall Objective: Assess the correlation between changes in AChR antibody level and clinical improvement by changes in the MG-ADL score and changes in QMG score.

Specific Aim 1: Assess the association between MG patients study group (ETTX vs. prednisone alone) and percent change in AChR antibody levels from baseline to 12, 24, and 36 months.

Specific Aim 1 Hypothesis: Myasthenic patients who received the thymectomy (ETTX), will have a greater percent change in antibody level from baseline to follow-up months (12, 24, 36), compared to prednisone alone patients.

Specific Aim 2: Assess the association between percent change in AChR antibody levels from baseline to 12, 24, 36 months and change MG-ADL & QMG scores at corresponding timepoints.

Specific Aim 2 Hypothesis: Greater reductions in antibody levels will result in clinical improvement; therefore, MG-ADL and QMG scores will decrease.

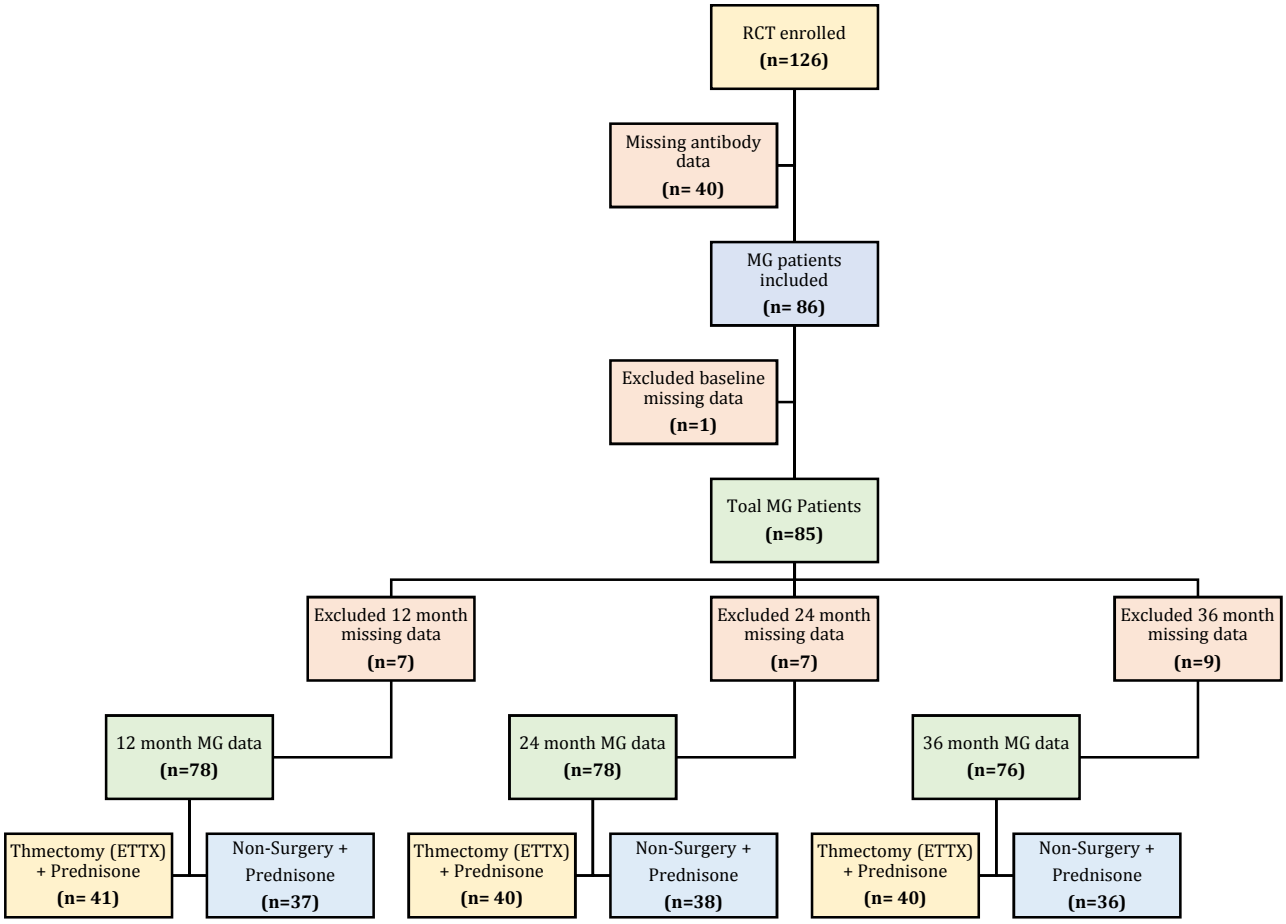
CHAPTER 2: METHODS

Study Design

A Post-hoc analysis of previously collected data from the phase III Thymectomy Trial in Non-Thymomatous Myasthenia Gravis Patients Receiving Prednisone Therapy (MGTX) (ClinicalTrials.gov identifier: NCT00294658) was performed. Changes in Myasthenia Gravis Activities of Daily Living (ΔMG-ADL) and changes in Quantitative Myasthenia Gravis (ΔQMG) scores were compared to changes in anti-acetylcholine receptor antibody levels (ΔAb), between the thymectomy+prednisone (ETTX) versus prednisone only groups. Antibody levels, MG-ADL, and QMG scores at baseline, 12, 24, and 36 months were analyzed. All data collection occurred during the MGTX clinical trial from 2015 through 2018, and with proper permissions, data was analyzed.

One hundred twenty-six (126) patients participated in the randomized controlled trial, but only 86 participants were identified for this research, as patients who had missing antibody levels (n=40) were excluded. Of these patients, only 85 were utilized, as participants who had missing bassline antibody levels (n=1) were also excluded. Pairwise deletion method was utilized to determine sample size of participants for the 3 timepoints: 12, 24, and 36 months (Figure 3). After excluding patients who had missing 12-month data (n=41) 78 patients were analyzed for the 12-month timepoint, of which (n=41) were in the ETTX study group and (n=37) were in the prednisone only group. Similarly, the 24-month timepoint included 78 subjects, (n=40) in the ETTX treatment group, and (n=38) in the prednisone only group. Lastly, the 36-month timepoint was comprised of 76 subjects, (n=40) who were in the ETTX study group, and (n=36) who were in the control study group.

***Figure 3**: Consort Flow Diagram for Baseline, 12, 24, 36 months data (pairwise deletion)*



Conceptual Models

As previously mentioned, this research aimed to determine the association between MG patients study group (thymectomy + prednisone vs. prednisone alone) and the percent change in acetylcholine receptor antibody levels from baseline to 12, 24, and 36 months. Figure 1 demonstrates the first conceptual model, where the primary exposure variable for this research question is the group the patient was randomized to: thymectomy+ prednisone (ETTX) or non-surgery/prednisone only. The outcome variable for this

question is the percent change in antibody levels from baseline to 12, 24, and 36 months, as seen in Figure 1.

This research also determined the association between percent change in antibody levels of patients from their baseline visit to their 12, 24, and 36 months visit to the changes in MG-ADL and QMG scores at the corresponding timepoints. Figure 2 shows that the primary exposure variable for the second research question is the percent change in antibody levels from baseline to 12, 24, and 36 months. The outcome variable for this question is the change in MG-ADL and QMG score from baseline to the corresponding timepoints, as seen in Figure 2.

The other independent variables that were included in this analysis are: age, sex, race, and ethnicity. In efforts to avoid multicollinearity, ethnicity was combined with race, leaving only 3 independent variables (age, sex, race/ethnicity "race"). Collinearity analysis was then performed to identify multicollinearity between variables in in the final model. The collinearity analysis resulted in all the independent variables being lower than 5, indicating that no multicollinearity occurred in the analysis. We believe that sex and age can both be potential covariate variables, as previous literatures has shown that females and younger aged MG patients have lower AChR antibody levels.[1] It is possible that being a younger female could impact the AChR antibody levels and impact the MG-ADL/QMG score, indicating that sex and age could be potential covariate variables in the relationship between the two questions proposed in this research. Sex and age were adjusted for in this analysis for this reason. Correspondingly, we believe race/ethnicity can be a covariate variables because we have learned that health dipartites exist amongst minority groups (Hispanics, Blacks, Other), thus race/ethnicity were also adjusted for in this analysis.

Figure 1: *Conceptual diagram of the association between MG patients study group (thymectomy vs. non-surgery/prednisone) and the percent change in antibody levels from baseline to 12, 24, 36 months.*

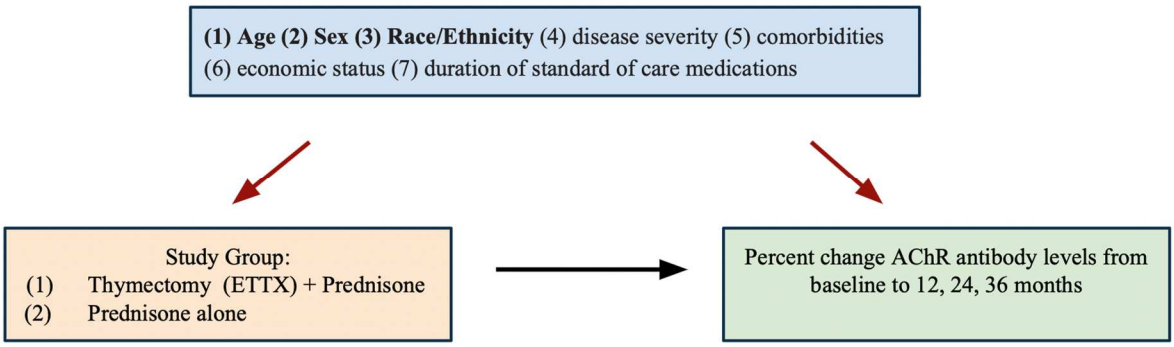
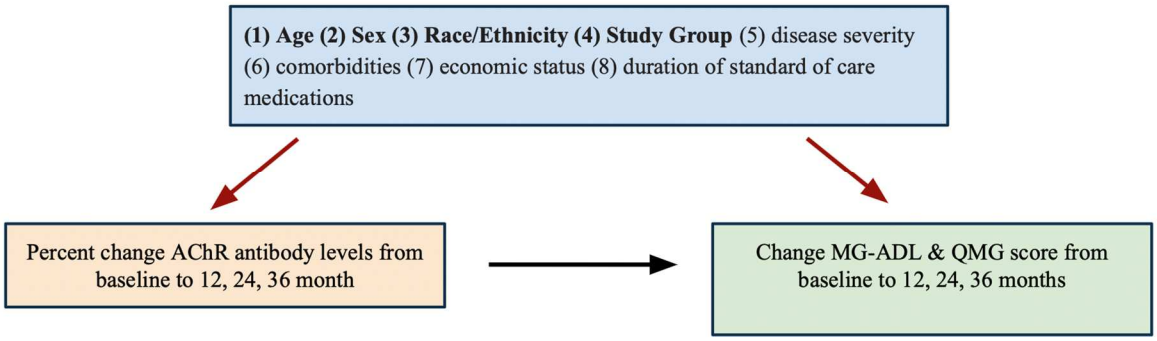


Figure 2: *Conceptual diagram of the association between percent change in antibody levels from baseline to 12, 24, 36 month and delta change MG-ADL score from baseline to 12, 24, 36 months in MG patients.*



Statistical Methods and Analysis

The data utilized for this research was collected from the clinical trial MGTX data set (ClinicalTrials.gov identifier: NCT00294658). The first research question's primary independent variable (MG patients study group) is considered a categorical variable. As where the first research question's dependent variable (the percent change in antibody level from baseline to 12, 24, & 36 months) is considered a continuous variable. For the second research question the independent variable (the change in antibody level from baseline to 12, 24, & 36 months) is considered a continuous variable. Similarly, the second research question's dependent variable (Change MG-ADL & QMG score from baseline to 12, 24, & 36 months) is also considered a continuous variable.

We performed descriptive statistics by calculating the mean, standard deviation, median, and range of continuous variables. For categorical variables, we calculated frequency counts and percentages. Percent Change in antibody level was calculated as a percent change from the baseline value, this was done by dividing the follow-up value by the baseline value. We performed bivariate analysis to estimate the association between primary exposure variables and our primary outcome variables. Our first research questions primary exposure variable was the study group, as where our second research questions primary exposure variable was the percent change in acetylcholine receptor antibody levels from baseline to 12, 24, & 36 months. Percent change in acetylcholine receptor antibody levels from baseline to 12, 24, & 36 months was our first research outcome variable, as where change MG-ADL & QMG score from baseline to 12, 24, & 36 months was our second research outcome variable.

We also utilized linear regression for both research questions. We included the following candidate variables based on the conceptual model: age, sex, race/ethnicity, and study group. Backwards method was utilized for variable selection, and we removed the variables with the highest p-value one by one, until we were left with only statistically significant p values. The variables with statistically significant p values were utilized for the final model. We performed Spearman Correlation to estimate the correlation between the percent change in antibody levels vs. change in MG-ADL & change in QMG scores. Study groups, ETTX and prednisone only, were calculated separately, but were also analyzed together (ETTX & prednisone only). Spearman correlation was utilized, as the data was skewed, and outliers were still included.[3] We then performed Mann-Whitney to estimate the difference between the study groups and confirm the results of the linear regression performed. This research analysis utilized a P value ≤ 0.05 to determine statistical significance. The above analyses were done using 2 statistical programs, (1) R, R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. (2) GraphPad Prism version 9.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

CHAPTER 3: Result

Patient Characteristics

As seen in Table 1 there was a total of 86 MG patients (N=86), 52.3% (N=45) were in the ETTX study group, 70.9% (N=61) were female, and the mean age was 35.6 years old. Table 1 also showed that 52.3% (N=45) of the participants were white, 26.7% (N=23) were Hispanic, 11.6% (N= 10) were Black/African American, and 9.3% (N=8) were considered other race/ethnicity.

Table 2 showed the Mean (SD) Baseline antibody level was 179 for the ETTX study group, and 342 for the prednisone only group. Table 2 also revealed that mean (SD) percent change in antibody levels for ETTX study group at 12-month was 85.7%, compared to prednisone group 95.2%. At 24-months the SD percent change in antibody levels for ETTX study group was 93.6,% and prednisone only was 96.4%. At the final timepoint, 36-months, the SD of percent change in antibody levels was 112% for the ETTX group, and 103% for the prednisone only group. Figure 4 demonstrated the mean percent change in antibody levels at baseline, 12, 24, 36 months for each study group (blue- ETTX, orange-Prednisone alone). Figure 4 showed that the percent antibody levels dropped at 12 months (ETTX=-14.3%, control=-4.8%), 24 months (ETTX=-6.4%, control=-3.6%), but increased at 36 months (ETTX=12%, control=3%).

Additionally, Table 2 also showed the Mean (SD) for the change in MG-ADL scores at Baseline, 12, 24, 36 months. The mean (SD) for the change in MG-ADL score from Baseline to the 3 timepoints are: 12 month (ETTX=-3.22, control=-1.85), 24 month (ETTX=-3.20, control=-1.85), and 36 month (ETTX=-3.26, control=-2.72). These values were presented in Figure 5, which showed that both study groups demonstrated reductions in MG-ADL score

at 12, 24, and 36 months. Finally, Table 2 showed the Mean (SD) for the change in QMG scores at Baseline, 12, 24, 36 months. The mean (SD) for the change in QMG score from Baseline to the 3 timepoints are: 12 month (ETTX=-5.04 control=-3.53), 24 month (ETTX=5.82, control=-3.62), and 36 month (ETTX=-6.21, control=-3.97). These values are visually presented in Figure 6, and demonstrated that the mean change in QMG dropped at 12, 24, and 36 months for both study groups.

Table 1: *Baseline patient characteristic of included myasthenia gravis patients. Note: ETTX is the thymectomy study group*

	ETTX (N=45)	Prednisone (N=41)	Overall (N=86)
Study Group			
ETTX	45 (100%)	0 (0%)	45 (52.3%)
Prednisone	0 (0%)	41 (100%)	41 (47.7%)
Age			
Mean (SD)	35.7 (13.3)	35.5 (12.2)	35.6 (12.7)
Median [Min, Max]	33.0 [18.0, 63.0]	33.0 [18.0, 63.0]	33.0 [18.0, 63.0]
Sex			
Female	33 (73.3%)	28 (68.3%)	61 (70.9%)
Male	12 (26.7%)	13 (31.7%)	25 (29.1%)
Race			
Black/African American	6 (13.3%)	4 (9.8%)	10 (11.6%)
Hispanic	12 (26.7%)	11 (26.8%)	23 (26.7%)
Other	3 (6.7%)	5 (12.2%)	8 (9.3%)
White, not Hispanic origin	24 (53.3%)	21 (51.2%)	45 (52.3%)

Figure 4: *Mean Percent change antibody levels at Baseline, 12, 24, and 36 months for each study group. Note: (1) Percent Change in antibody levels at 12, 24, 36 months was calculated as a percent change from the baseline value, this was done by dividing the follow-up value by the baseline value. (2) For both study groups, all three timepoints were not statistically significant*

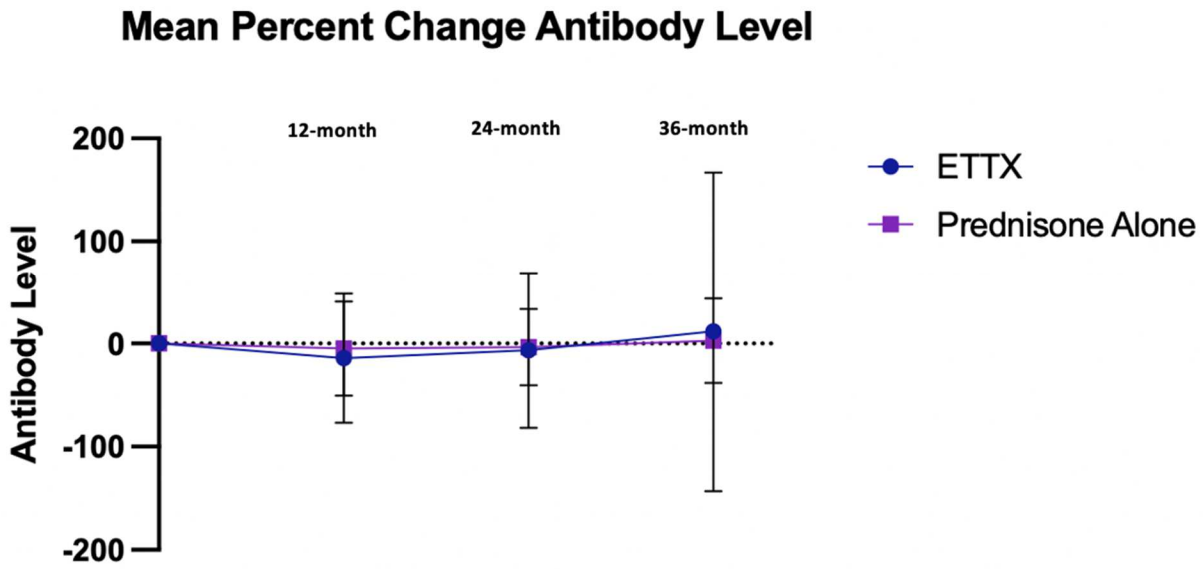


Table 2: *Mean values for Antibody levels, MG-ADL and QMG scores at baseline, 12, 24, 36 month of included myasthenia gravis patients. Note: (1) ETTX is the thymectomy +prednisone study group. (2) Percent Change in antibody levels at 12, 24, 36 months was calculated as a percent change from the baseline value, this was done by dividing the follow-up value by the baseline value.*

	ETTX (N=45)	Prednisone (N=41)	Overall (N=86)
Baseline Antibody Levels			
Mean (SD)	179 (371)	342 (766)	257 (595)
Median [Min, Max]	28.6 [0.250, 1680]	53.9 [1.02, 3820]	31.9 [0.250, 3820]
Percent change in antibody levels at 12 months			
Mean (SD)	85.7 (62.8)	95.2 (45.8)	90.3 (55.2)
Median [Min, Max]	85.3 [10.3, 398]	97.0 [1.37, 258]	90.1 [1.37, 398]
Missing	4 (8.9%)	3 (7.3%)	7 (8.1%)
Percent change in antibody levels at 24 months			
Mean (SD)	93.6 (75.5)	96.4 (37.1)	95.0 (59.4)
Median [Min, Max]	77.7 [4.50, 384]	99.9 [20.9, 184]	97.3 [4.50, 384]
Missing	5 (11.1%)	2 (4.9%)	7 (8.1%)
Percent change in antibody levels at 36 months			
Mean (SD)	112 (155)	103 (41.0)	108 (115)
Median [Min, Max]	74.1 [1.37, 920]	103 [23.7, 280]	92.4 [1.37, 920]
Missing	4 (8.9%)	4 (9.8%)	8 (9.3%)
Baseline MG-ADL score			
Mean (SD)	5.38 (3.13)	5.18 (3.12)	5.28 (3.11)
Median [Min, Max]	5.00 [0, 13.0]	4.50 [1.00, 12.0]	5.00 [0, 13.0]
Missing	0 (0%)	1 (2.4%)	1 (1.2%)
Change in MG-ADL at 12 months			
Mean (SD)	-3.22 (3.50)	-1.85 (2.82)	-2.58 (3.26)
Median [Min, Max]	-3.00 [-10.0, 4.00]	-1.50 [-9.00, 3.00]	-2.00 [-10.0, 4.00]
Missing	0 (0%)	1 (2.4%)	1 (1.2%)
Change in MG-ADL at 24 months			
Mean (SD)	-3.20 (3.30)	-2.36 (3.20)	-2.81 (3.26)
Median [Min, Max]	-3.00 [-11.0, 6.00]	-2.00 [-10.0, 3.00]	-3.00 [-11.0, 6.00]
Missing	1 (2.2%)	2 (4.9%)	3 (3.5%)
Change in MG-ADL at 36 months			
Mean (SD)	-3.26 (3.30)	-2.72 (2.96)	-3.00 (3.13)
Median [Min, Max]	-3.00 [-13.0, 6.00]	-3.00 [-8.00, 3.00]	-3.00 [-13.0, 6.00]
Missing	2 (4.4%)	2 (4.9%)	4 (4.7%)
Baseline QMG score			
Mean (SD)	11.0 (4.82)	12.2 (5.10)	11.5 (4.96)
Median [Min, Max]	11.0 [1.00, 21.0]	11.0 [5.00, 22.0]	11.0 [1.00, 22.0]
Missing	0 (0%)	1 (2.4%)	1 (1.2%)
Change in QMG at 12 months			
Mean (SD)	-5.04 (4.90)	-3.53 (4.36)	-4.33 (4.69)
Median [Min, Max]	-4.00 [-19.0, 7.00]	-3.50 [-15.0, 7.00]	-4.00 [-19.0, 7.00]
Missing	0 (0%)	1 (2.4%)	1 (1.2%)
Change in QMG at 24 months			
Mean (SD)	-5.82 (5.09)	-3.62 (4.34)	-4.78 (4.85)
Median [Min, Max]	-6.00 [-19.0, 4.00]	-3.00 [-12.0, 7.00]	-4.00 [-19.0, 7.00]
Missing	1 (2.2%)	2 (4.9%)	3 (3.5%)
Change in QMG at 36 months			
Mean (SD)	-6.21 (5.31)	-3.97 (4.86)	-5.15 (5.19)
Median [Min, Max]	-6.00 [-19.0, 4.00]	-4.00 [-15.0, 5.00]	-5.50 [-19.0, 5.00]
Missing	2 (4.4%)	2 (4.9%)	4 (4.7%)

Figure 5: *Mean Change MG-ADL scores at Baseline, 12, 24, and 36 months for each study group. Note: (*) indicates statistical significance.*

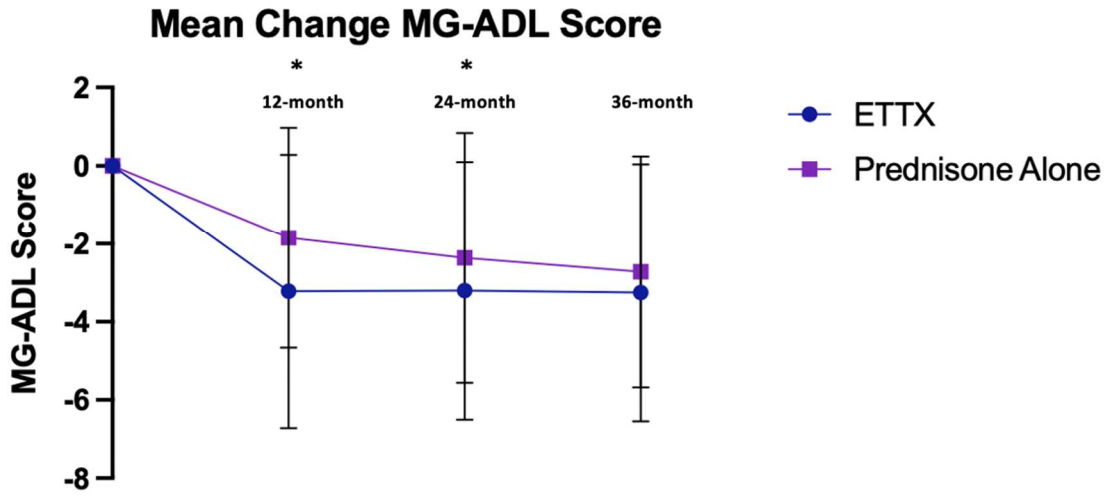
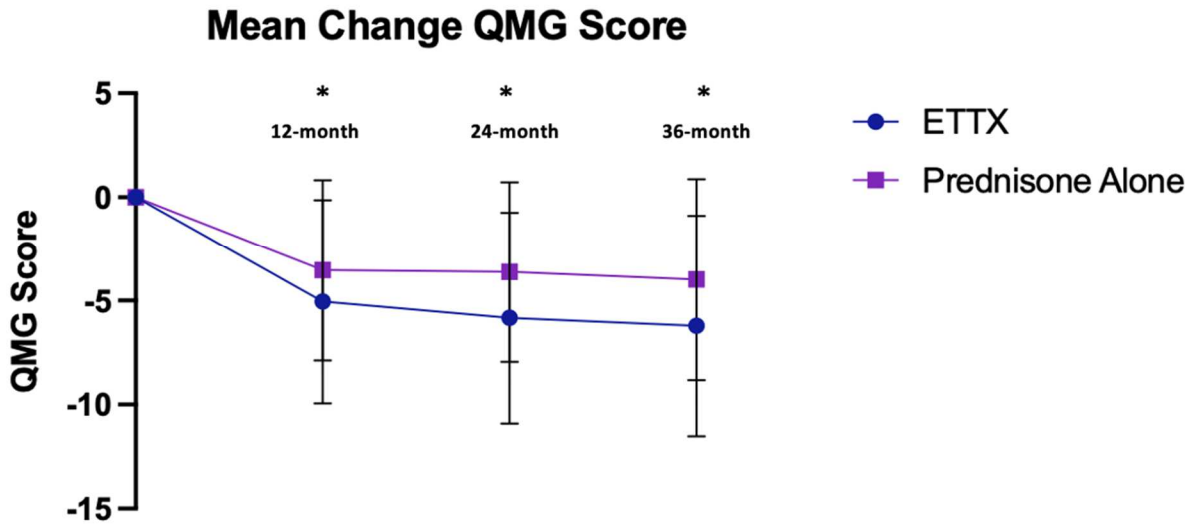


Figure 6: *Mean Change QMG scores at Baseline, 12, 24, and 36 months for each study group. Note: (*) indicates statistical significance.*



Results by Specific Aim

***Specific Aim 1*:** Assess the association between MG patients study group (ETTX vs. prednisone alone) and percent change in AChR antibody levels from baseline to 12, 24, and 36 months.

Specific Aim 1 Hypothesis: Myasthenic patients who received the thymectomy (ETTX), will have a greater percent change in antibody level from baseline to follow-up months (12, 24, 36), compared to prednisone alone patients.

Results: Specific aim 1 results are shown in Tables 3-5. The association between study group and percent change in AChR antibody from baseline was analyzed at 12, 24, and 36 months (Table 3, Table 4, Table 5, correspondingly). Bivariate analysis revealed that the association between study group and percent change in AChR antibody level does not exist, due to all p values being greater than 0.05 (p=0.45, p=0.80, p=0.67, respectively).

For the first research question, after running the backwards method for the multivariate model, we found that none of the independent variables were left in the final model, due to all the independent variables p values being greater than 0.05. The final model we were left with, only included the primary independent variable (study group); therefore, our final model was the bivariate model (Tables 3-5). Tables 3, 4, and 5 demonstrated the association between MG patients study group (ETTX vs. prednisone alone) and the percent change in AChR antibody levels from baseline to 12, 24, 36 months does not exist, due to the p values ((p=0.45, p=0.80, p=0.67, respectively), being greater than 0.05.

Table 3: *The association between study group and percent change in antibody level from baseline to 12 months*

Characteristic	N	Beta	95% CI[1]	p-value
Study Group	78			
ETTX		—	—	
Prednisone		9.6	-16, 35	0.45
Age	78	-0.16	-1.1, 0.82	0.74
Sex	78			
Female		—	—	
Male		-17	-45, 11	0.22
Race	78			
Black/African American		—	—	
Hispanic		19	-25, 62	0.39
Other		19	-34, 72	0.48
White, not Hispanic origin		38	-2.7, 78	0.067

[1] CI = Confidence Interval

Table 4: *The association between study group and percent change in antibody level from baseline to 24 months*

Characteristic	N	Beta	95% CI[1]	p-value
Study Group	78			
ETTX		—	—	
Prednisone		3.5	-24, 31	0.80
Age	78	-0.17	-1.2, 0.88	0.74
Sex	78			
Female		—	—	
Male		-33	-63, -2.4	0.035
Race	78			
Black/African American		—	—	
Hispanic		-10	-59, 38	0.68
Other		-62	-121, -3.9	0.037
White, not Hispanic origin		-18	-63, 27	0.44

[1] CI = Confidence Interval

Table 5: *The association between study group and percent change in antibody level from baseline to 36 months*

Characteristic	N	Beta	95% CI[1]	p-value
Study Group	76			
ETTX		—	—	
Prednisone		-12	-65, 42	0.67
Age	76	0.87	-1.2, 2.9	0.40
Sex	76			
Female		—	—	
Male		-40	-100, 21	0.19
Race	76			
Black/African American		—	—	
Hispanic		-89	-189, 10	0.078
Other		-129	-252, -6.5	0.039
White, not Hispanic origin		-86	-180, 8.5	0.074

[1] CI = Confidence Interval

Specific Aim 2: Assess the association between percent change in AChR antibody levels from baseline to 12, 24, 36 months and change MG-ADL & QMG scores at corresponding timepoints.

Specific Aim 2 Hypothesis: Greater reductions in antibody levels will result in clinical improvement; therefore, MG-ADL and QMG scores will decrease.

Results: Specific aims 2 results are shown in Tables 6-8 for changes in MG-ADL score, and Tables 9-11 for changes in QMG score. Additionally, specific aim 2 results are revealed in Figures 7, 9, 11 for changes in MG-ADL, and Figures 8, 10, 12 for changes in QMG. Finally, specific aim 2 results are revealed in Tables 12, 13, 16, 17, 20, and 21 for changes in MG-ADL, and Tables 14, 15, 18, 19, 22, and 23 for changes in QMG.

When running the backwards method for the multivariate model for the second research question, none of the independent variables were left in the final model, due to all the independent variables p values being greater than 0.05. The final model we were left with, only included the primary independent variable (percent change in antibody levels); therefore, our final model was also a bivariate model (Tables 6-11).

Specific Aim 2: Changes in MG-ADL

Table 6 showed that the association between percent change in antibody level from baseline to 12 month and change in MG-ADL score is statically significant, due to p value ($p = <0.001$). The Beta coefficient ($B=0.01$) revealed that a 100 unit increase in percent change in antibody level is associated with a 2-point increase in MG-ADL score. Table 6 also reveals that the independent variables (Study Group, Age, Sex, Race/Ethnicity) are not sufficient covariates, due to all p values being greater than 0.05. Table 7 displayed the association between percent change in antibody level from baseline to 24 month and change in MG-ADL score is not statically significant, due to p value being greater than 0.05 ($p = 0.08$). Similarly, Table 8 showed that an association between percent change in antibody level at 36 months and change in MG-ADL score is not statistically significant, due to the p value ($p=0.089$).

Figure 7 demonstrated the correlation between the percent change in antibody levels and change in MG-ADL at 12, 24, and 36 months, when study groups were separated (ETTX vs. prednisone alone). Figure 7 revealed that the correlation was not statistically significant at 12, 24, and 36 months for the ETTX group, due to the P values being greater than 0.05 at all 3 timepoints ($p=0.164$, $p=0.2331$, $p=0.144$, respectively). Similarly, Figure 7 showed that a correlation was not identified at timepoints 24 and 36

months for the prednisone only group, due to the P values (p=0.166, p=350, respectively). Additionally, Figure 7 showed that a correlation between the percent change in antibody levels and change in MG-ADL was present at the 12-month timepoint (P=0.009). The correlation 0 [5]

Figure 9 demonstrated the correlation between the percent change in antibody levels and change in MG-ADL at 12, 24, and 36 months, when study groups were combined (ETTX + prednisone alone). Figure 9 revealed that a correlation between the percent change in antibody levels and change in MG-ADL was only statistically significant at the 12 and 24 month timepoint (p=0.0008, p=0.0397, correspondingly). The R values revealed that the strength of this correlation was considered moderate for both the 12 and 24 month timepoint, (R=0.373, R=0.0397, respectively). Finally, Figure 11 demonstrated the correlation of percent change in antibody level and change in MG-ADL at 12, 24, and 36 months for all graphs analyzed. Figure 11 combines figure 7 and figure 9, so that the graphs may be analyzed side by side.

***Specific Aim 2:** Changes in QMG*

Tables 9-11 demonstrated the association between percent change in antibody level at 12, 24, 36 months and change in QMG score was statistically significant, due to p values (p=0.044, p=0.012, p=0.018, correspondingly) being less than 0.05. Table 9 and 10 had the same Beta coefficient (B=0.02), which revealed that a 100 unit increase in percent change in antibody level is associated with a 2-point increase in QMG score. Similarly, Table 11 Beta coefficient (B=0.01) revealed that a 100 unit increase in percent change in antibody level is associated with a 1-point increase in QMG score.

Figure 8 demonstrated the correlation between the percent change in antibody levels and change in QMG at 12, 24, and 36 months, when study groups were separated (ETTX vs. prednisone alone). Figure 8 revealed that a correlation between the percent change in antibody levels and change in QMG for the ETTX group was only statistically significant at the 12 and 36 month timepoint (p=0.0213, p=0.0386, correspondingly). The R values revealed that the strength of this correlation was considered moderate for both the 12 and 36 month timepoint (R=0.358, R=0.328, respectively). Figure 8 also showed that the correlation for the prednisone alone group was only statistically significant at the 24 and 36 month timepoint (p=0.020, p=0.0135, correspondingly). The R values revealed that the strength of this correlation was considered moderate for both the 24 and 36 month timepoint (R=0.376, R=0.408, respectively).

Figure 10 demonstrated the correlation between the percent change in antibody levels and change in QMG at 12, 24, and 36 months, when study groups were combined (ETTX + prednisone alone). Figure 10 revealed the correlation was statistically significant at 12, 24, and 36 months, due to the P values being less than 0.05 at all 3 timepoints (p=0.0032, p=0.0031, p=0.0005, respectively).The R values revealed a moderate strength of correlation at 12 month (R=0.329), and 24 months (R=0.331), and at 36 months (R=0.391). Finally, Figure 12 demonstrated the correlation of percent change in antibody level and change in QMG at 12, 24, and 36 months for all graphs analyzed. Figure 12 combines figure 8 and figure 10, so that the graphs may be analyzed side by side.

Lastly, Man-Whitney testing (Tables 12-17) was performed to confirm that the samples collected are likely to originate from the same population. Man-Whitney U testing was performed, as our assumption was of sample independence. Tables 12, 16, and 17

initially revealed statistically significant p values (p=0.0259, p=0.00929, p=0.00929, correspondingly), but due to having multiples timepoints (12, 24, and 36 months) Bonferroni correction was then performed. Bonferroni correction corrected the p value for multiple comparisons and revealed that statistical significance would be determined at 0.0083. Tables 12, 16, and 17 all had p values greater than 0.0083; therefore, none of the Man-Whitney U testing was statistically significant.

Table 6: *The association between percent change in antibody level and delta change in MG-ADL from baseline to 12 months*

Characteristic	N	Beta	95% CI[1]	p-value
Change12m.Antibody	78	0.02	0.01, 0.04	<0.001
Study Group	78			
ETTX		—	—	
Prednisone		1.2	-0.30, 2.7	0.12
Age	78	-0.02	-0.08, 0.04	0.46
Sex	78			
Female		—	—	
Male		-0.50	-2.2, 1.2	0.56
Race	78			
Black/African American		—	—	
Hispanic		2.2	-0.37, 4.7	0.093
Other		-0.85	-4.0, 2.3	0.59
White, not Hispanic origin		1.6	-0.82, 4.0	0.19

[1] CI = Confidence Interval

Table 7: *The association between percent change in antibody level and delta change in MG-ADL from baseline to 24 months*

Characteristic	N	Beta	95% CI[1]	p-value
Change24m.Antibody	78	0.01	0.00, 0.02	0.080
Study Group	78			
ETTX		—	—	
Prednisone		0.88	-0.61, 2.4	0.24
Age	78	0.01	-0.05, 0.06	0.84
Sex	78			
Female		—	—	
Male		-0.38	-2.1, 1.3	0.67
Race	78			
Black/African American		—	—	
Hispanic		0.38	-2.3, 3.1	0.78
Other		-2.0	-5.3, 1.3	0.23
White, not Hispanic origin		0.54	-2.0, 3.1	0.67

[1] CI = Confidence Interval

Table 8: *The association between percent change in antibody level and delta change in MG-ADL from baseline to 36 months*

Characteristic	N	Beta	95% CI[1]	p-value
Change36m.Antibody	76	0.01	0.00, 0.01	0.089
Study Group	76			
ETTX		—	—	
Prednisone		0.50	-1.0, 2.0	0.50
Age	76	0.01	-0.05, 0.07	0.80
Sex	76			
Female		—	—	
Male		-0.02	-1.7, 1.7	0.98
Race	76			
Black/African American		—	—	
Hispanic		0.48	-2.4, 3.3	0.74
Other		0.29	-3.2, 3.8	0.87
White, not Hispanic origin		0.80	-1.9, 3.5	0.56

[1] CI = Confidence Interval

Table 9: *The association between percent change in antibody level and delta change in QMG from baseline to 12 months*

Characteristic	N	Beta	95% CI[1]	p-value
Change12m.Antibody	78	0.02	0.00, 0.04	0.044
Study Group	78			
ETTX		—	—	
Prednisone		1.4	-0.70, 3.6	0.18
Age	78	0.00	-0.08, 0.09	0.92
Sex	78			
Female		—	—	
Male		-2.0	-4.3, 0.39	0.10
Race	78			
Black/African American		—	—	
Hispanic		2.0	-1.5, 5.4	0.26
Other		-3.8	-8.1, 0.41	0.076
White, not Hispanic origin		2.7	-0.54, 5.9	0.10

[1] CI = Confidence Interval

Table 10: *The association between percent change in antibody level and delta change in QMG from baseline to 24 months*

Characteristic	N	Beta	95% CI[1]	p-value
Change24m.Antibody	78	0.02	0.01, 0.04	0.012
Study Group	78			
ETTX		—	—	
Prednisone		2.1	-0.09, 4.2	0.060
Age	78	0.06	-0.02, 0.15	0.16
Sex	78			
Female		—	—	
Male		-2.2	-4.7, 0.29	0.083
Race	78			
Black/African American		—	—	
Hispanic		0.70	-3.2, 4.6	0.72
Other		-4.1	-8.8, 0.54	0.082
White, not Hispanic origin		1.5	-2.1, 5.1	0.41

[1] CI = Confidence Interval

Table 11: *The association between percent change in antibody level and delta change in QMG from baseline to 36 months*

Characteristic	N	Beta	95% CI[1]	p-value
Change36m.Antibody	76	0.01	0.00, 0.02	0.018
Study Group	76			
ETTX		—	—	
Prednisone		2.3	-0.06, 4.6	0.056
Age	76	0.04	-0.06, 0.13	0.44
Sex	76			
Female		—	—	
Male		-1.7	-4.3, 1.0	0.22
Race	76			
Black/African American		—	—	
Hispanic		1.2	-3.3, 5.7	0.59
Other		-1.1	-6.7, 4.4	0.68
White, not Hispanic origin		1.4	-2.9, 5.6	0.52

[1] CI = Confidence Interval

Figure 7: *Spearman Correlation comparing ETTX to Prednisone only (Percent Change in Antibody level vs Change in MG-ADL)*

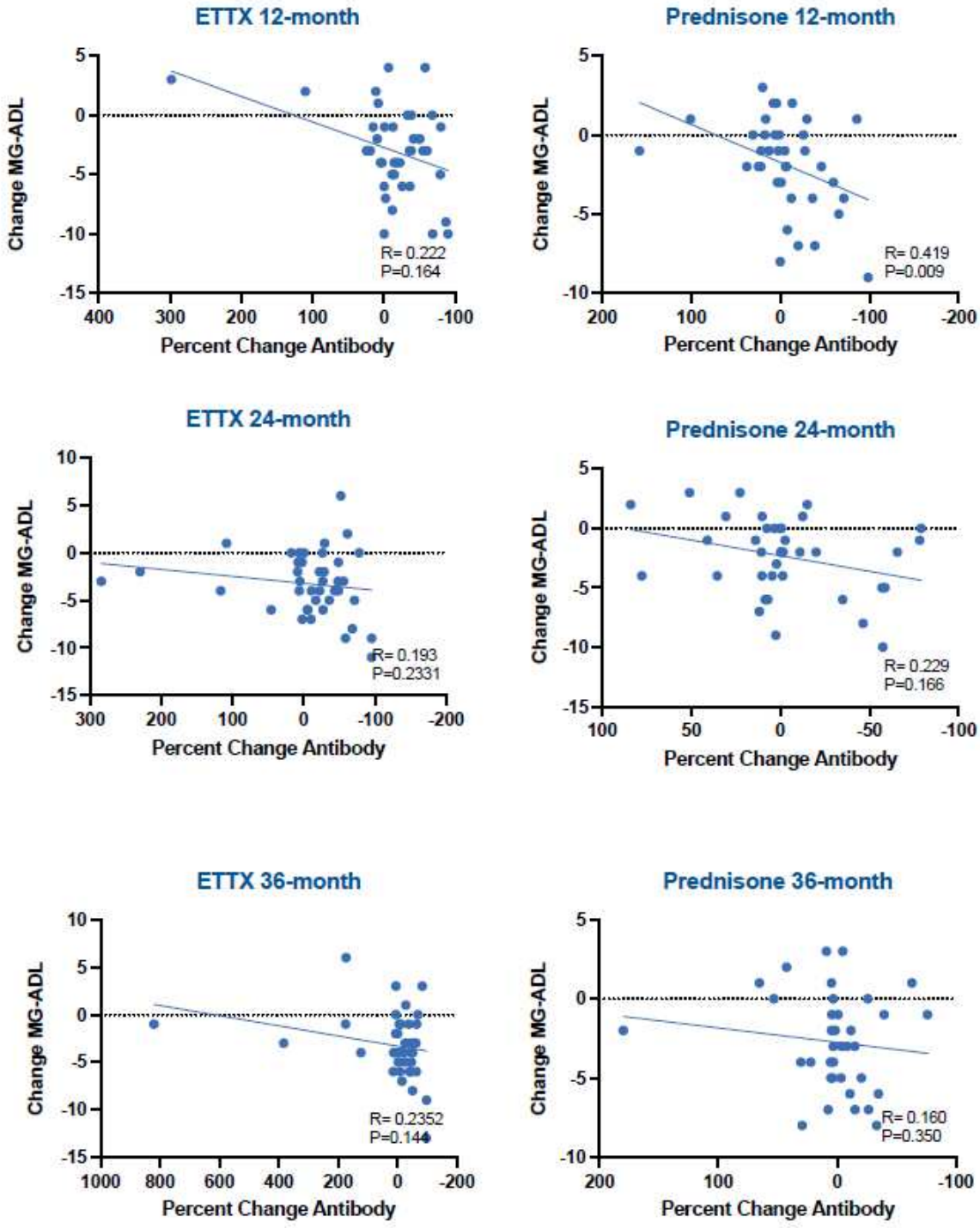


***Figure 8**: Spearman Correlation comparing ETTX to Prednisone only (Percent Change in Antibody level vs Change in QMG)*

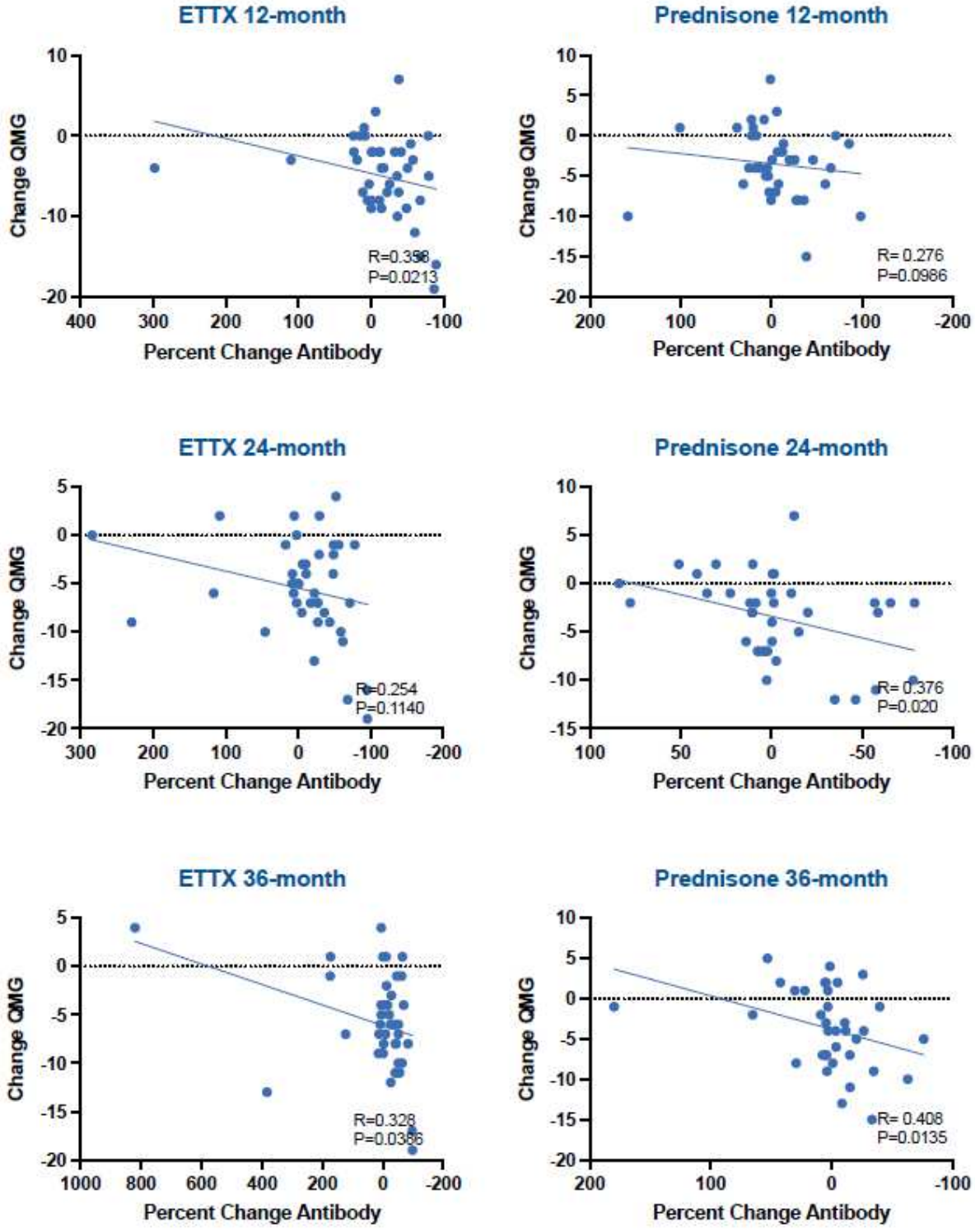
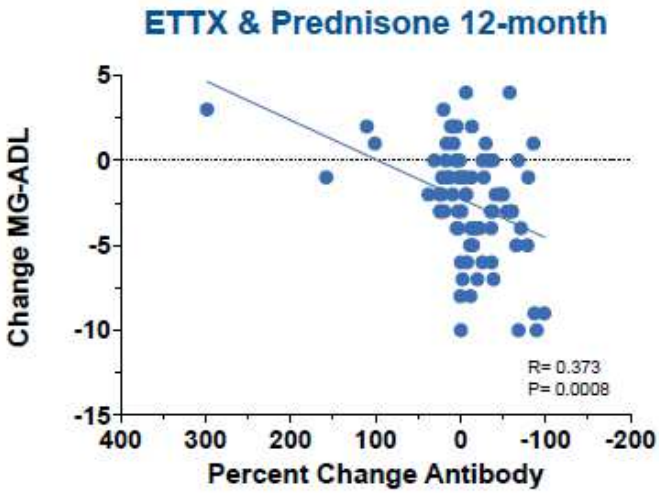
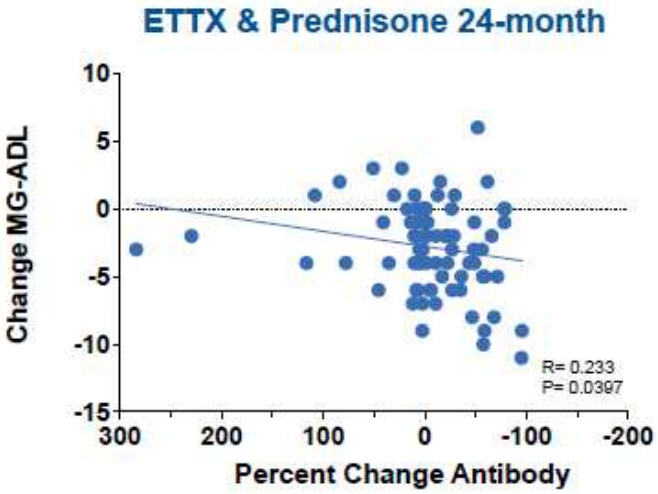


Figure 9: *Spearman Correlation of ETTX & Prednisone only (Percent Change in Antibody level vs Change in MG-ADL)*





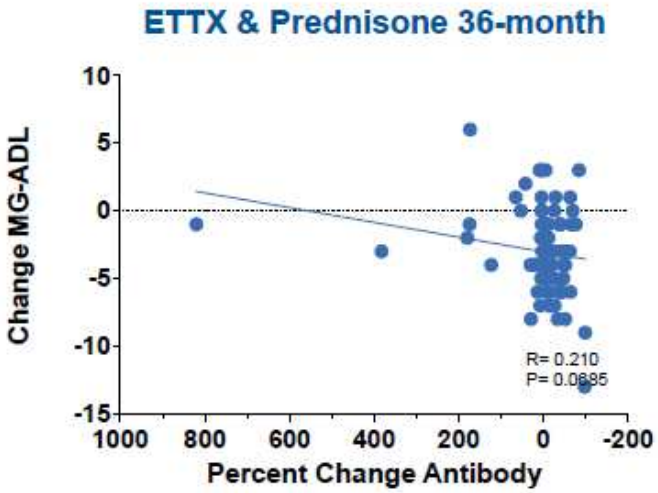


Figure 10: *Spearman Correlation of ETTX & Prednisone only (Percent Change in Antibody level vs Change in QMG)*

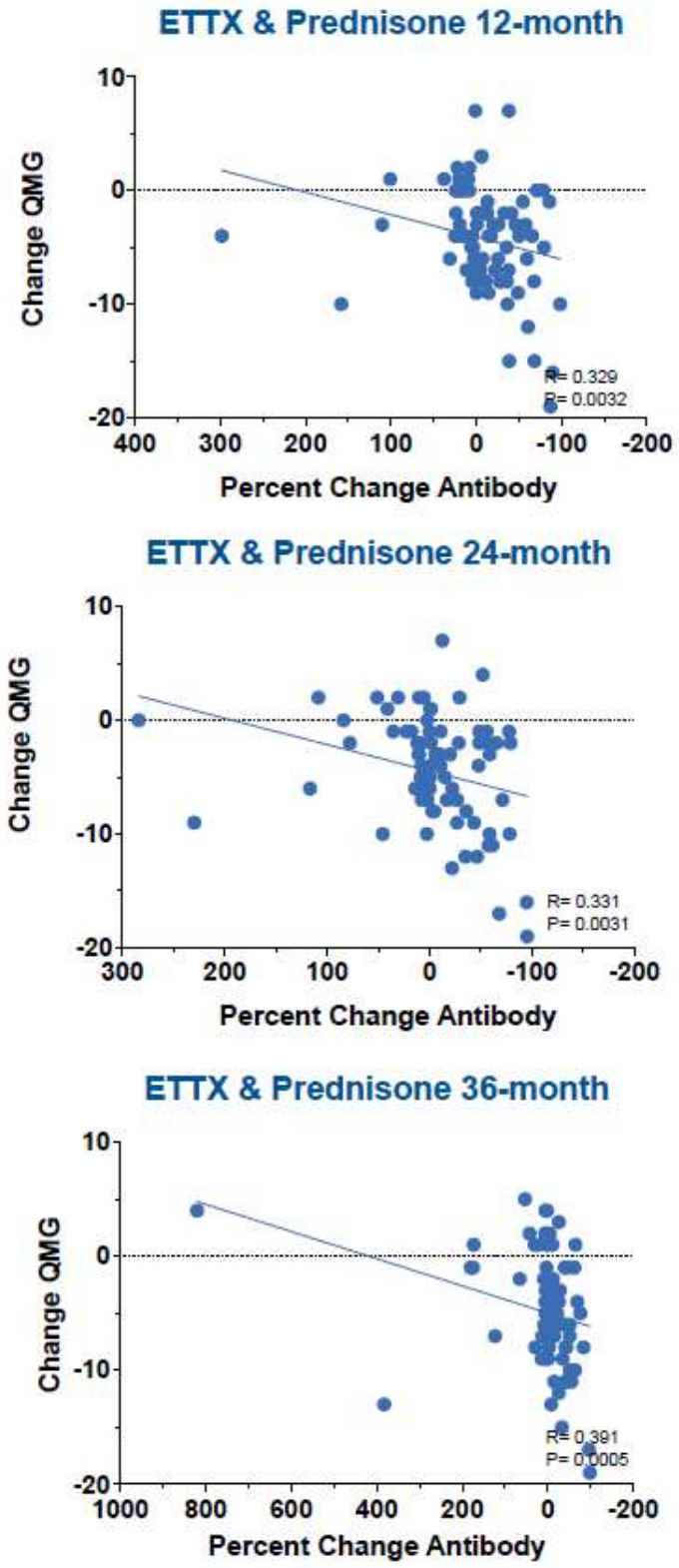


Figure 11: *Spearman Correlation showing all graphs, ETTX, Prednisone only, and ETTX & Prednisone, (Percent Change in Antibody level vs Change in MG-ADL)*

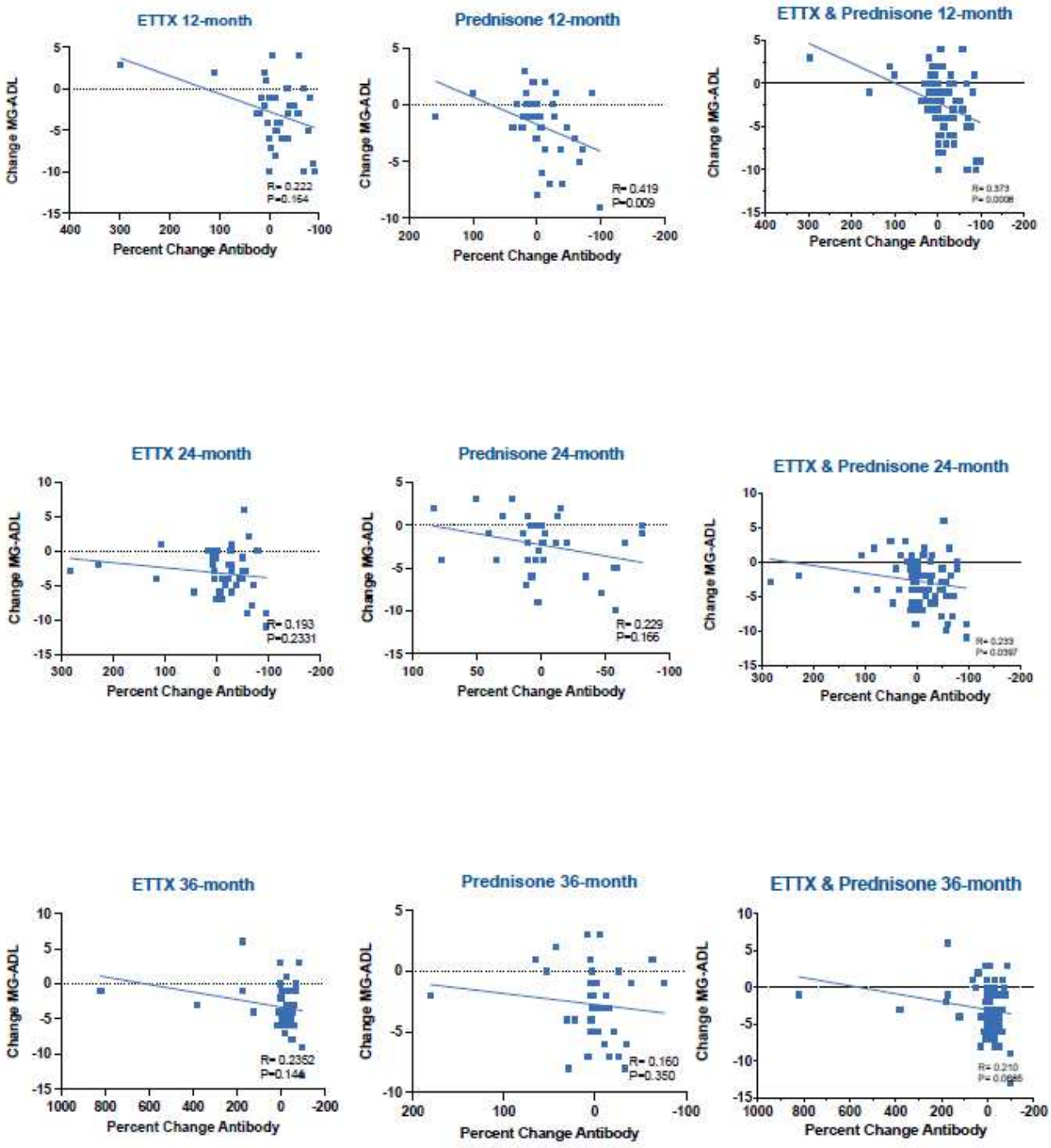


Figure 12: *Spearman Correlation showing all graphs, ETTX, Prednisone only, and ETTX & Prednisone, (Percent Change in Antibody level vs Change in QMG)*

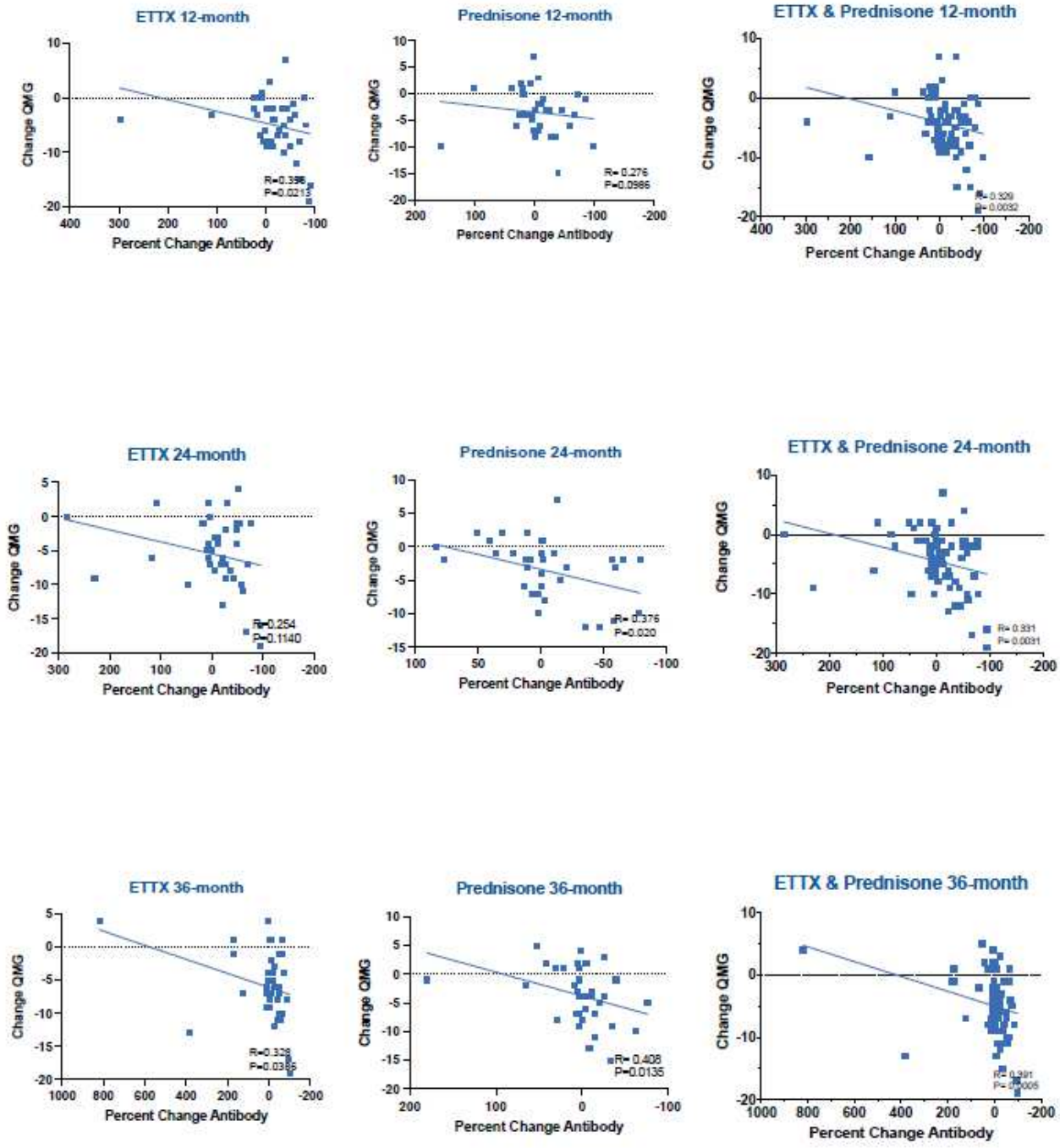


Table 12: *Mann-Whitney test for 12-month Percent change antibody and change MG-ADL*

Multiple Mann-Whitney tests of 12m ADL

	Discovery?	P value	Mean rank of ETTX	Mean rank of Prednisone	Mean rank diff.	Mann-Whitney U	q value
Percent Change Antibody 12m	No	0.079827	35.63	44.71	-9.076	600.0	0.080625
ADL Change12m	No	0.025983	37.42	49.28	-11.85	649.0	0.052486

Table 13: *Mann-Whitney test for 12-month Percent change antibody and change QMG*

Multiple Mann-Whitney tests of 12m QMG

	Discovery?	P value	Mean rank of ETTX	Mean rank of Pred	Mean rank diff.	Mann-Whitney U	q value
Percent Change Antibody 12m	No	0.079827	35.63	44.71	-9.076	600.0	0.161251
QMG Change12m	No	0.171190	39.54	46.89	-7.343	744.5	0.172902

Table 14: *Mann-Whitney test for 24-month Percent change antibody and change MG-ADL*

Multiple Mann-Whitney tests of 24m ADL

	Discovery?	P value	Mean rank of ETTX	Mean rank of Prednisone	Mean rank diff.	Mann-Whitney U	q value
Percent Change Antibody 24m	No	0.060143	35.20	44.92	-9.723	588.0	0.121488
ADL Change24m	No	0.171984	38.60	45.83	-7.231	708.5	0.173704

Table 15*: Mann-Whitney test for 24-month Percent change antibody and change QMG*

Multiple Mann-Whitney tests of 24m QMG

	Discovery?	P value	Mean rank of ETTX	Mean rank of Prednisone	Mean rank diff.	Mann-Whitney U	q value
Percent Change Antibody 24m	No	0.060143	35.20	44.92	-9.723	588.0	0.060744
QMG Change24m	No	0.058859	37.31	47.29	-9.988	651.5	0.060744

Table 16: *Mann-Whitney test for 36-month Percent change antibody and change MG-ADL*

Multiple Mann-Whitney tests of 36m ADL

	Discovery?	P value	Mean rank of ETTX	Mean rank of Prednisone	Mean rank diff.	Mann-Whitney U	q value
Percent Change Antibody 36m	No	0.009295	33.20	46.49	-13.29	500.0	0.018776
ADL Change36m	No	0.417161	39.47	43.74	-4.278	751.0	0.421332

Table 17: *Mann-Whitney test for 36-month Percent change antibody and change QMG*

Multiple Mann-Whitney tests of 36m QMG

	Discovery?	P value	Mean rank of ETTX	Mean rank of Prednisone	Mean rank diff.	Mann-Whitney U	q value
Percent Change Antibody 36m	No	0.009295	33.20	46.49	-13.29	500.0	0.018776
QMG Change36m	No	0.062105	36.84	46.64	-9.804	638.0	0.062726

CHAPTER 4: Discussion

Discussion by Specific Aims:

Specific Aim 1: Assess the association between MG patients study group (ETTX vs. prednisone alone) and the percent change in AChR antibody levels from baseline to 12, 24, and 36 months.

Hypothesis: Myasthenic Patients who received the thymectomy, will have a greater reduction in percent change in antibody level from baseline to follow-up month (12, 24, 36), compared to prednisone alone patients.

Results: Specific aim 1 results are shown in Tables 3-5. The association between study group and percent change in AChR antibody from baseline was analyzed at 12, 24, and 36 months (Table 3, Table 4, Table 5, correspondingly). The p values for all 3 timepoints revealed that the association for between study group and percent change in AChR antibody level does not exist, due to all p values being greater than 0.05 ($p=0.45$, $p=0.80$, $p=0.67$, respectively).

Discussion: In analysis of results for specific aim 1, the research uncovered that an association between study group and percent change in AChR antibody levels from baseline to 12, 24, and 36 months did not exist. These results suggest that the percent change in antibody levels from baseline to the three timepoints is not clearly associated with the study group patients were randomized to. Both study groups received prednisone. Taken together, these results suggest that there is no clear impact of ETTX on the percent change in antibody levels at 12, 24, and 36 months for MG patients.

Specific Aim 2: Assess the association between percent change in AChR antibody levels from baseline to 12, 24, 36 months and change MG-ADL & QMG scores at corresponding timepoints.

Specific Aim 2 Hypothesis: Greater reductions in antibody levels will result in clinical improvement; therefore, MG-ADL and QMG scores will decrease.

Results: Specific aims 2 results are shown in Tables 6-8 for changes in MG-ADL score, and Tables 9-11 for changes in QMG score. Additionally, specific aim 2 results are revealed in Figures 7, 9, 11 for changes in MG-ADL, and Figures 8, 10, 12 for changes in QMG. Finally, specific aim 2 results are shown in Tables 12, 13, 16, 17, 20, and 21 for changes in MG-ADL, and Tables 14, 15, 18, 19, 22, and 23 for changes in QMG.

Discussion for changes in MG-ADL: Results of the bivariate analysis for the 12-month timepoint (Table 6) suggest that for every 100 unit increase in percent change in antibody level, there is a 2-point increase in MG-ADL score. These results propose that as antibody level increases by 100%, MG patients' clinical status worsens, as MG-ADL score increase by 2-points, and greater scores embody greater severity of symptoms. Results of the spearman correlation (Figure 9) suggested that when study groups were combined a moderate correlation between the percent change in antibody levels and change in MG-ADL score were seen at the 12 and 24 month timepoint. The 12 and 24 month timepoint graphs depicted on Figure 9 demonstrate that a greater percentage drop in AChR antibody levels correlates with greater decrease in MG-ADL scores. These results suggest that MG clinical status improves with greater drops in AChR antibody levels. A two-point improvement (decrease in score) in MG-ADL is highly significant from the clinical standpoint and has been the primary discriminatory outcome in pivotal trials of recently approved therapies in

MG. [25] One of the recently approved therapies is Efgartigimod (ARGX-113), and the study showed that treated patients demonstrated a clinically meaningful improvement (≥ 2 point improvement) in MG-ADL score for 48.7% of the time between start of study till week 18, compared with 26.6% of the same period in the placebo group. [25] Ultimately, a 2-point improvement in MG-ADL is considered clinically meaningful.

Discussion for changes in QMG: Results of the bivariate analysis (Tables 9-10) also suggest all timepoints demonstrated a statistically significant association between percent change in AChR antibody levels from baseline to 12, 24, 36 months and change QMG scores exists. The B coefficients for the 12 and 24 month (Table 9 and Table 10) timepoint revealed that for every 100 unit increase in percent change in antibody level, there is a 2-point increase in QMG score. Similarly, bivariate analysis also suggested that for the 36-month timepoint (Table 11) that for every 100 unit increase in percent change in antibody level, there is a 1-point increase in QMG score. These results propose that as antibody level increase by 100%, MG patients' clinical status worsen, as the QMG score increased by 1 to 2-points, and greater scores represent greater severity of symptoms. Results of the spearman correlation (Figure 10) suggested that when study groups were combined a moderate correlation between the percent change in antibody levels and change in QMG score were seen at all 3 timepoints timepoint. The 12, 24, 36 month timepoint graphs depicted on Figure 10 demonstrate that a greater percentage drop in AChR antibody levels correlates with greater decrease in QMG scores. These results suggest that MG clinical status improves with greater drops in AChR antibody levels. A 2-point change in QMG score is not considered clinically meaningful, a change of at least 3 points needs to be identified to be considered clinically meaningful. Although the changes

in QMG were not considered clinically meaningful, the QMG score results still showed that QMG corresponded with the reduction in AChR antibody levels at all 3 timepoints.

The Use of AChR Antibody Levels as a Biomarker for Disease Severity:

Although no statistical difference was demonstrated between the two study groups in terms of percent change in AChR antibody levels, both subgroups demonstrated a drop in AChR antibody, and this is considered clinically meaningful. In current practice, AChR antibodies are solely used for diagnostic purposes, but the drop in AChR antibody seen in both treatment arms suggests these antibody titers may serve for more than just diagnosing myasthenia gravis. Ultimately, the proposed research revealed that reductions in AChR antibody level correlated in both treatment arms, with improvement in QMG and MG-ADL scores. This statistically significant correlation suggests that AChR antibodies should be analyzed more carefully, and not only used for diagnostic purposes. The research demonstrated clinical improvement correlated with reductions in AChR antibody levels; therefore, AChR antibodies could serve as a biomarker for disease severity in MG. If AChR antibodies were used as an intermediate response variable, clinicians could use these antibody levels to monitor treatment effectiveness in MG patients. Overall, the use of AChR antibody levels as a biomarker for disease severity in MG would truly change how MG patients are treated today, and hopefully result in patients living a better quality of life.

Several previous and ongoing MG clinical trials are utilizing drug mechanisms that result in reductions in AChR antibody titers. [24] The most common mechanism of action of these drugs are (1) neonatal Fc receptor (FcRn) inhibitors (2) B-cell depletion therapies, either through CD19 or CD20 receptor antagonism or plasma cell depletion (through

CD38 depletion). [24] FcRn inhibitors remove AChR antibodies, as where B-cell therapies prevent AChR antibody production. [24] Examples of these types of drugs include: efgartigimod (FcRN antagonist), rozanolixizumab (FcRn antagonist), inebilizumab (CD19 depletion), and rituximab (CD20 depletion), but there are numerous other new drugs in the pipeline, including TAK-079 (a plasma cell antagonist). [24] Despite the varying mechanisms of action, both drug mechanisms ultimately result in reductions in AChR antibodies. Previous and ongoing clinical trials are utilizing drug mechanisms that result in the reduction of AChR antibodies to investigate if these drugs result in MG clinical improvement. The proposed research demonstrated a statistically significant correlation between AChR antibody reduction and clinical improvement in MG patients; therefore, this research supports the AChR reducing therapies for myasthenia gravis. Nonetheless further evaluation of the correlation between AChR antibody levels and clinical status needs to be conducted, and guided by the data shown in this research, we would like to propose a prospective analysis to further investigate this correlation in the new upcoming thymectomy trial for ocular myasthenia gravis.

Importance of Serial Serum Dilutions:

It is important to note that the AChR antibody samples collected in the MGTX clinical trial were analyzed with careful serial dilutions. Recently published in 2021, Dr. Angela Vincent argued that the usefulness in measuring antibodies is only present if the antibody levels are calculated accurately and carefully, by dedicating serial serum dilutions. [23] Similarly, Investigators Lazaridis and Tzartos suggested that obtaining sequential samples of autoantibodies of the same MG patient adds to monitoring and managing their disease.[16] The moderate correlation seen in the percent change in antibody levels and

change in MG-ADL and QMG score may be a result of the proper serial dilutions performed when processing the AChR antibody samples. Additionally, In 2021, Investigators Masuda *et al.* conducted a retrospective cohort study, to analyze the clinical relevance in MG with their developed assay that detects autoantibodies against the main immunogenic region of the AChR.[20] The researchers found that antibody levels of the main immunogenic region of the AChR were a greater indicator than the routinely used AChR binding antibody[20] These recent publications support the argument that the assay of AChR antibody samples is essential for its usefulness to managing and monitoring patients clinical status.

Limitations:

While the prospectively collected data is considered a strength in this analysis, the post-hoc nature inherently has its limitations. A principal limitation of this study was that only 68% (n=86) of patient's enrolled data was analyzed in this study; therefore, there is potential for sampling bias. Secondly, pot-hoc data analyses can propose the issue of new discoveries being nothing more than a coincidence, due to post-hoc studies not following the randomization model of statistical inference. [7] It is possible that the correlation seen in this analysis is purely coincidence, due to this study being a post-hoc data analysis.

Secondly, the MGTX clinical trial did not have a true placebo group, as no true sham procedure of an ETTX was performed. Although no sham procedure was performed, the study made all efforts to blind the clinical evaluator by providing turtlenecks to all patients so that the clinicians wouldn't see the surgical scar and be unblinded to if patients received the thymectomy or not. Nonetheless, not having a true placebo is a limitation within this research.

Additionally, another significant limitation of the present study was only having 3 independent variables (age, sex, race/ethnicity). Other potential covariates that should have been considered but were not provided include disease severity and other comorbidities that patients' may have. The variables could potentially result in a biased analysis, due to disease severity and other comorbidities potential for impacting the exposure or outcome variables analyzed. Future studies should consider adjusting for these potential candidate variables.

Lastly, a principal limitation of this study was small sample size. As mentioned previously, 32% (n=40) of patient's data was not utilized, as this data was completely missing from the MGTX clinical trial. Due to small sample size, outliers were included in the final analysis, and this increased variability in the analysis.

Future Directions

Finally, there are evident steps that need to be taken before anti-acetylcholine antibody levels can be integrated into clinical use as a biomarker for disease activity in MG patients. First, a sensitivity analysis of the data presented needs to be conducted. Due to the small samples size, this research was analyzed with outliers included. A sensitivity analysis would identify the variation that exists within the data and allow for outliers to be identified and eliminated, resulting in a more accurate interpretation of the data.

Additionally, running a generalized estimating equation (GEE) with autoregressive integrated moving average (ARIMA) should be performed. This research analyzed the change in antibody level and change in MG-ADL and QMG scores over time from baseline to 12, 24, and 36 months. Conducting the GEE with ARIMA would allow researchers to

describe the changes with Antibody levels, MG-ADL scores, and QMG scores over the 3 years.[10]

Lastly, this study should be conducted with a larger sample size of myasthenia gravis patients. This research utilized the captured data of a previous clinical trial to analyze the correlation between antibody levels and clinical improvement. As mentioned in the limitations, the sample size for this study was extremely small, as several patients (n=40) data was missing. Furthermore, the correlation between antibody levels and clinical improvement in MG patients should be conducted as a multicenter prospective study in the future. As mentioned previously a new thymectomy trial for ocular myasthenia gravis will be starting this year, we would like to propose a prospective analysis to investigate the AChR antibodies in the new thymectomy trial. Conducting this research with a greater population and as a prospective study would potentially further our scientific knowledge of the correlation between anti-acetylcholine receptor antibody levels and MG clinical response.

CHAPTER 5: Summary and Conclusions

The relationship of anti-acetylcholine receptor antibody levels to treatment response remains unclear in seropositive myasthenia gravis patients. Today, AchR antibody levels are used purely for establishing MG diagnosis. This research analysis revealed that reductions in AChR antibody level correlated in both treatment arms, with improvement in QMG and MG-ADL scores. Improvement in MG-ADL score was statistically significant at 12 and 24 months (P= 0.0397 and P=0.0008 respectively) and demonstrated a moderate correlation (R=0.373, R=0.233 correspondingly). Additionally, improvement in QMG score and reductions in antibody level directly correlated at all 3 timepoints [P= 0.0032, P= 0.0031, P=0.0005, respectively], with a moderate correlation [R=0.329, R=0.331, R=0.331 correspondingly]. This statistically significant correlation between reductions in AChR antibody level and clinical improvement suggests the use of AChR levels as a biomarker for disease activity in MG patients. However, further evaluation of the correlation between AChR antibody level and clinical response needs to be conducted, as it may provide additional information about implementing AChR antibody levels as a biomarker for disease activity in MG patients.

References

[1] Aurangzeb, Sidra, Muhammad Tariq, Muhammad Irshad, Mazhar Badshah, and Rao Suhail Y Khan. "Relationship between Anti-Acetylcholine Receptor Antibody Titres and Severity of Myasthenia Gravis." *J Pak Med Assoc*, n.d., 3.

[2] Baets, M. H. de, and Henricus Josephus Gerardus Hubertus Oosterhuis. *Myasthenia Gravis*, 2019. https://search.ebscohost.com/login.aspx?direct=true&scope=site&db=nlebk&db=nlabk&AN=2154140.

[3] Bishara, Anthony J., and James B. Hittner. "Testing the Significance of a Correlation with Nonnormal Data: Comparison of Pearson, Spearman, Transformation, and Resampling Approaches." *Psychological Methods* 17, no. 3 (September 2012): 399–417. https://doi.org/10.1037/a0028087.

[4] Carr, Aisling S, Chris R Cardwell, Peter O McCarron, and John McConville. "A Systematic Review of Population Based Epidemiological Studies in Myasthenia Gravis." *BMC Neurology* 10, no. 1 (December 2010): 46. https://doi.org/10.1186/1471-2377-10-46.

[5] Chan, Y H. "Biostatistics 104: Correlational Analysis," n.d., 6.

[6] CHEESE study group, Cheng-Che Chou, I-Chen Su, I-Jun Chou, Jainn-Jim Lin, Shih-Yun Lan, Yi-Shan Wang, et al. "Correlation of Anti-Acetylcholine Receptor Antibody Levels and Long-Term Outcomes of Juvenile Myasthenia Gravis in Taiwan: A Case Control Study." *BMC Neurology* 19, no. 1 (December 2019): 170. https://doi.org/10.1186/s12883-019-1397-0.

[7] Curran-Everett, Douglas, and Henry Milgrom. "Post-Hoc Data Analysis: Benefits and Limitations." *Current Opinion in Allergy & Clinical Immunology* 13, no. 3 (June 2013): 223–24. https://doi.org/10.1097/ACI.0b013e3283609831.

[8] Galassi, Giuliana, Marco Mazzoli, Alessandra Ariatti, Shaniko Kaleci, Franco Valzania, and Paolo F. Nichelli. "Antibody Profile May Predict Outcome in Ocular Myasthenia Gravis." *Acta Neurologica Belgica* 118, no. 3 (September 2018): 435–43. https://doi.org/10.1007/s13760-018-0943-7.

[9] Gilhus, Nils Erik, Geir Olve Skeie, Fredrik Romi, Konstantinos Lazaridis, Paraskevi Zisimopoulou, and Socrates Tzartos. "Myasthenia Gravis — Autoantibody Characteristics and Their Implications for Therapy." *Nature Reviews Neurology* 12, no. 5 (May 2016): 259–68. https://doi.org/10.1038/nrneurol.2016.44.

[10] Heldal, Anne Taraldsen, Geir Egil Eide, Fredrik Romi, Jone Furlund Owe, and Nils Erik Gilhus. "Repeated Acetylcholine Receptor Antibody-Concentrations and Association to Clinical Myasthenia Gravis Development." Edited by Güher Saruhan-Direskeneli. *PLoS ONE* 9, no. 12 (December 2, 2014): e114060. https://doi.org/10.1371/journal.pone.0114060.

[11] Hong, Yu, Xiao Liang, and Nils Erik Gilhus. "AChR Antibodies Show a Complex Interaction with Human Skeletal Muscle Cells in a Transcriptomic Study." *Scientific Reports* 10, no. 1 (December 2020): 11230. https://doi.org/10.1038/s41598-020-68185-x.

[12] Howard, Frank M., Vanda A. Lennon, Jon Finley, Joseph Matsumoto, and Lila R. Elveback. "Clinical Correlations of Antibodies That Bind, Block, or Modulate Human Acetylcholine Receptors in Myasthenia Gravis." *Annals of the New York Academy of Sciences* 505, no. 1 Myasthenia Gr (August 1987): 526–38. https://doi.org/10.1111/j.1749-6632.1987.tb51321.x.

[13] Kang, Sa-Yoon, Jung-Hwan Oh, Sook Keun Song, Jung Seok Lee, Jay Chol Choi, and Ji-Hoon Kang. "Both Binding and Blocking Antibodies Correlate with Disease Severity in Myasthenia Gravis." *Neurological Sciences* 36, no. 7 (July 2015): 1167–71. https://doi.org/10.1007/s10072-015-2236-8.

[14] Kojima, Yuta, Akiyuki Uzawa, Yukiko Ozawa, Manato Yasuda, Yosuke Onishi, Hiroyuki Akamine, Naoki Kawaguchi, et al. "Rate of Change in Acetylcholine Receptor Antibody Levels Predicts Myasthenia Gravis Outcome." *Journal of Neurology, Neurosurgery & Psychiatry* 92, no. 9 (September 2021): 963–68. https://doi.org/10.1136/jnnp-2020-325511.

[15] Koneczny, Inga, and Ruth Herbst. "Myasthenia Gravis: Pathogenic Effects of Autoantibodies on Neuromuscular Architecture." *Cells* 8, no. 7 (July 2, 2019): 671. https://doi.org/10.3390/cells8070671.

[16] Lazaridis, Konstantinos, and Socrates J. Tzartos. "Autoantibody Specificities in Myasthenia Gravis; Implications for Improved Diagnostics and Therapeutics." *Frontiers in Immunology* 11 (February 14, 2020): 212. https://doi.org/10.3389/fimmu.2020.00212.

[17] Li, Lihua, Meaghan S Cuerden, Bian Liu, Salimah Shariff, Arsh K Jain, and Madhu Mazumdar. "Three Statistical Approaches for Assessment of Intervention Effects: A Primer for Practitioners." *Risk Management and Healthcare Policy* Volume 14 (February 2021): 757–70. https://doi.org/10.2147/RMHP.S275831.

[18] Lindstrom, Jon M., Marjorie E. Seybold, Vanda A. Lennon, Senga Whittingham, and Drake D. Duane. "Antibody to Acetylcholine Receptor in Myasthenia Gravis: Prevalence, Clinical Correlates, and Diagnostic Value." *Neurology* 51, no. 4 (October 1998): 933.2-933-a. https://doi.org/10.1212/WNL.51.4.933-a.

[19] Marcuse, Florit, Lloyd Brandts, Daan Moens, Jan Damoiseaux, Monique Hochstenbag, Janneke G. J. Hoeijmakers, Jos G. Maessen, and Marc De Baets. "The Association between Anti-acetylcholine Receptor Antibody Level and Clinical Improvement in Myasthenia Gravis." *European Journal of Neurology* 29, no. 4 (April 2022): 1187–97. https://doi.org/10.1111/ene.15238.

[20] Masuda, Tomoko, Masakatsu Motomura, Kimiaki Utsugisawa, Yuriko Nagane, Ruka Nakata, Masahiro Tokuda, Taku Fukuda, Toshiro Yoshimura, Mitsuhiro Tsujihata, and Atsushi Kawakami. "Antibodies against the Main Immunogenic Region of the Acetylcholine Receptor Correlate with Disease Severity in Myasthenia Gravis." *Journal of Neurology, Neurosurgery & Psychiatry* 83, no. 9 (September 2012): 935–40. https://doi.org/10.1136/jnnp-2012-302705.

[21] Sanders, Donald B., Ted M. Burns, Gary R. Cutter, Janice M. Massey, Vern C. Juel, Lisa Hobson-Webb, and The Muscle Study Group. "Does Change in Acetylcholine Receptor Antibody Level Correlate with Clinical Change in Myasthenia Gravis?: Predictive Value of AChR Antibodies." *Muscle & Nerve* 49, no. 4 (April 2014): 483–86. https://doi.org/10.1002/mus.23944.

[22] Seybold, Marjorie E., and Jon M. Lindstrom. "PATTERNS OF ACETYLCHOLINE RECEPTOR ANTIBODY FLUCTUATION IN MY ASTHENIA GRAVIS." *Annals of the New York Academy of Sciences* 377, no. 1 Myasthenia Gr (December 1981): 292–306. https://doi.org/10.1111/j.1749-6632.1981.tb33739.x.

[23] Vincent, Angela. "Using AChR Antibody Titres to Predict Treatment Responses in Myasthenia Gravis." *Journal of Neurology, Neurosurgery & Psychiatry* 92, no. 9 (September 2021): 915–915. https://doi.org/10.1136/jnnp-2021-326480.

[24] Habib, Ali Aamer, Ghazaleh Ahmadi Jazi, and Tahseen Mozaffar. "Update on Immune□mediated Therapies for Myasthenia Gravis." *Muscle & Nerve* 62, no. 5 (November 2020): 579–92. https://doi.org/10.1002/mus.26919.

[25] Howard, James F, Vera Bril, Tuan Vu, Chafic Karam, Stojan Peric, Temur Margania, Hiroyuki Murai, et al. "Safety, Efficacy, and Tolerability of Efgartigimod in Patients with Generalised Myasthenia Gravis (ADAPT): A Multicentre, Randomised, Placebo-Controlled, Phase 3 Trial." *The Lancet Neurology* 20, no. 7 (July 2021): 526–36. https://doi.org/10.1016/S1474-4422(21)00159-9.